46. A method of modulating levels of an FGF-like polypeptide in an animal comprising administering to the animal the nucleic acid molecule of Claim 8.

Please cancel claims 6 and 14-35 without prejudice or disclaimer.

REMARKS

The Examiner indicated that claims 1-5, 7-13, 36, and 39-43 were pending at the issuance of the instant Office Action. Claims 1, 2, 12, 13, 36, 39, and 40 have been amended and new claims 44-46 have been added. Non-elected Claims 6 and 14-35 have been canceled without prejudice in favor of a divisional application. The amendments to the claims and new claims are fully supported by the specification. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

1. Rejection of claims 1-5, 7-13, 36, and 39-43 under 35 U.S.C. § 101

The Examiner rejected claims 1-5, 7-13, 36, and 39-43 under 35 U.S.C. § 101 because the claimed invention lacks patentable utility. The Examiner notes that the specification fails to disclose a functional assay demonstrating the asserted FGF-like activity of the claimed sequences and argues that in the absence of such data, the claimed invention lacks a "specific, well-established, and credible utility." Applicants traverse this rejection.

Applicants contend that the instant application contains an assertion of a specific and substantial utility for the claimed invention that one of ordinary skill in the art would find to be credible. The instant application discloses murine FGF-like nucleotide sequences that were isolated from a mouse regenerating liver cDNA library using a kFGF signal trap system, and human FGF-like nucleotide sequences that were isolated from a human liver cDNA library using a probe derived from the murine FGF-like gene. Nearly all of the related amino acid sequences identified in a BLAST search using the human FGF-like amino acid sequence (SEQ ID NO: 2) were found to be members of the FGF family of proteins (Exhibit A). Exhibit B illustrates that the instantly-claimed human FGF-like polypeptide is most closely related, but not identical, to an FGF (FGF-21) that is most preferentially expressed in the liver (Exhibit C; Nishimura *et al.*, 2000, *Biochim. Biophys. Acta*

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1492:203-06).

The instant application discloses that, in view of the localization of FGF-like mRNA expression (primarily in the liver), the structural similarity of FGF-like polypeptide to members of the FGF family, and the likelihood that FGF-like polypeptide is secreted into the bloodstream where it may exert effects on distal sites, the FGF-like molecules of the present invention may be useful for, *inter alia*, stimulating cells within or near the liver, regulating intestinal cell activity, or stimulating pancreatic beta islet cells (page 5, lines 3-14). The instant application further discloses that transgenic mice expressing an FGF-like transgene of the invention exhibit an abnormal phenotype generally characterized as inhibited or delayed maturation, including reduced body weight, reduced liver weight as a percent of body weight, reduced spleen weight as percent of body weight, increased thymic weight as percent of body weight (possibly due to delayed thymic maturation and involution), and poorly developed ovaries with lack of significant follicular development (page 4, lines 22-28). Applicants contend that a skilled artisan would recognize that the claimed sequences could be useful, for example, as growth or fat deposition inhibitors (page 5, lines 15-16) or in the treatment or prevention of liver-related diseases and disorders (page 5, lines 23-25).

Applicants contend that because the instant application contains an assertion of a specific and substantial utility for the claimed invention that one of ordinary skill in the art would find to be credible, the rejection under 35 U.S.C. § 101 should be withdrawn.

2. Rejections of claims 39 and 40 under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 39 and 40 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner again notes that the specification fails to disclose a functional assay demonstrating the asserted FGF-like activity of the claimed sequences and argues that the specification therefore does not indicate what distinguishing feature is shared by the claimed genus of FGF-like nucleotide sequence variants, homologous sequences, and fragments. The Examiner further argues that because the functional limitation of exhibiting "FGF-like polypeptide activity" is insufficient to describe the genus of FGF-like nucleotide sequence variants, homologous

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sequences, and fragments, Applicants were not in possession of the claimed genus. Applicants traverse this rejection.

The instant application discloses an FGF-like polypeptide that is primarily expressed in the liver, thereby distinguishing it from *all* other members of the FGF family (page 4, line 39 to page 5, line 1). The instant application further discloses that transgenic mice expressing an FGF-like transgene exhibit an abnormal phenotype generally characterized as inhibited or delayed maturation. Applicants contend that the localization of FGF-like mRNA expression in the liver and the effect of FGF-like transgene expression on transgenic mice maturation sufficiently describe the genus of FGF-like nucleotide sequence variants, homologous sequences, and fragments. Therefore, Applicants respectfully request that this rejection be withdrawn.

3. Rejections of claims 1-5, 7-13, 36, and 39-43 under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 1-5, 7-13, 36, and 39-43 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention.

The Examiner notes that neither the specification nor the pending claims disclose an ATCC deposit number. A deposit of cDNA encoding FGF-like polypeptide, in the *E. coli* strain DH10B, was made by Applicants with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, before the filing date of the instant application. A copy of the ATCC receipt for this deposit, showing the patent deposit designation (Accession No. PTA-626) and the date on which the deposit was received by the ATCC (September 3, 1999), is attached. Applicants have amended the specification and claims accordingly, and contend that all the requirements of 37 C.F.R. 1.801 *et seq.* have now been met. *In re Lundak,* 225 U.S.P.Q. 90 (Fed. Cir. 1985).

The Examiner also argues that because the specification fails to disclose a specific biological function for the claimed sequences and the claimed sequences display a low sequence identity to members of the FGF family of proteins, a skilled artisan would not know how to use the claimed sequences or their variants or homologous sequences, or how to test for compounds that inhibit FGF-

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like polypeptide activity. Applicants contend that, based on the localization of FGF-like mRNA expression in the liver and the effect of FGF-like transgene expression on transgenic mice maturation, a skilled artisan would know how to use the claimed sequences or their variants or homologous sequences, or how to test for compounds that inhibit FGF-like polypeptide activity. Therefore, Applicants respectfully request that this rejection be withdrawn.

The Examiner further argues that the specification fails to provide an enabling disclosure for using the claimed sequences to modulate the levels of a polypeptide in an animal, as the claimed method reads on the modulation of any endogenous gene or polypeptide. The Examiner also argues that the potential therapies for practicing the claimed method – *e.g.*, antisense gene therapy – are highly unpredictable and would therefore, require undue experimentation. Applicants have amended claim 36 to indicate that the claimed sequences can be used to modulate the level of an FGF-like polypeptide, rather than of *any* endogenous gene or polypeptide. Applicants contend that one with skill in the art could readily practice the claimed method using, for example, antisense inhibitors identified by the genetic suppressor element (GSE) approach disclosed in U.S. Patent Nos. 5,217,889 and 5,811,234 (to Roninson *et al.*). Therefore, Applicants respectfully request that this rejection be withdrawn.

4. Rejections of claims 8, 9, 12, 13, 36, and 40 under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 8, 9, 12, 13, 36, and 40 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention.

The Examiner argues that claim 39 is unclear as to the meaning of the phrase "wherein the polypeptide has an activity of the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4." Applicants contend that since a skilled artisan could readily generate fragments of the claimed sequences of claim 39(a) that lack "an activity of the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4," the use of this phrase in claim 39(c) is not redundant. Applicants respectfully request that this rejection be withdrawn.

The Examiner also argues that claim 40(d) is unclear as to the meaning of phrase "C- and/or -terminal." Applicants thank the Examiner for pointing out the absence of the "N" in "N-terminal,"

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which as been added by amendment herein. Applicants contend that their amendment of claim 40 overcomes the asserted rejection, and request that it be withdrawn.

The Examiner objected to claims 8, 9, 12, 13, and 36 under 37 C.F.R. 1.75(c) as being of improper dependent form. Applicants have amended claims 12, 13, and 36 to place these claims into proper dependent form. However, Applicants are uncertain about the basis for the Examiner's objection to claims 8 and 9, and respectfully request that the Examiner provide a more precise explanation regarding the deficiencies thereof.

CONCLUSIONS

Applicants believe that the rejections under 35 U.S.C. § 112, first paragraph and § 112, second paragraph, have been overcome by amendment. Applicants further contend that the rejections under 35 U.S.C. § 101 and § 112, second paragraph, should be withdrawn, as the claimed sequences of the instant application have specific, substantial, and credible utility, and one with skill in the art would readily recognize how to make and use the claimed sequences. Allowance of the claims is thereby respectfully solicited.

If Examiner Stroup believes it to be helpful, she is invited to contact the undersigned representative by telephone at (312) 913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Dated: July 5, 2001

By: _____
Donald L. Zuhn, Ph.D.
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EXHIBIT A

BLASTP 2.1.3 [Apr-11-2001]

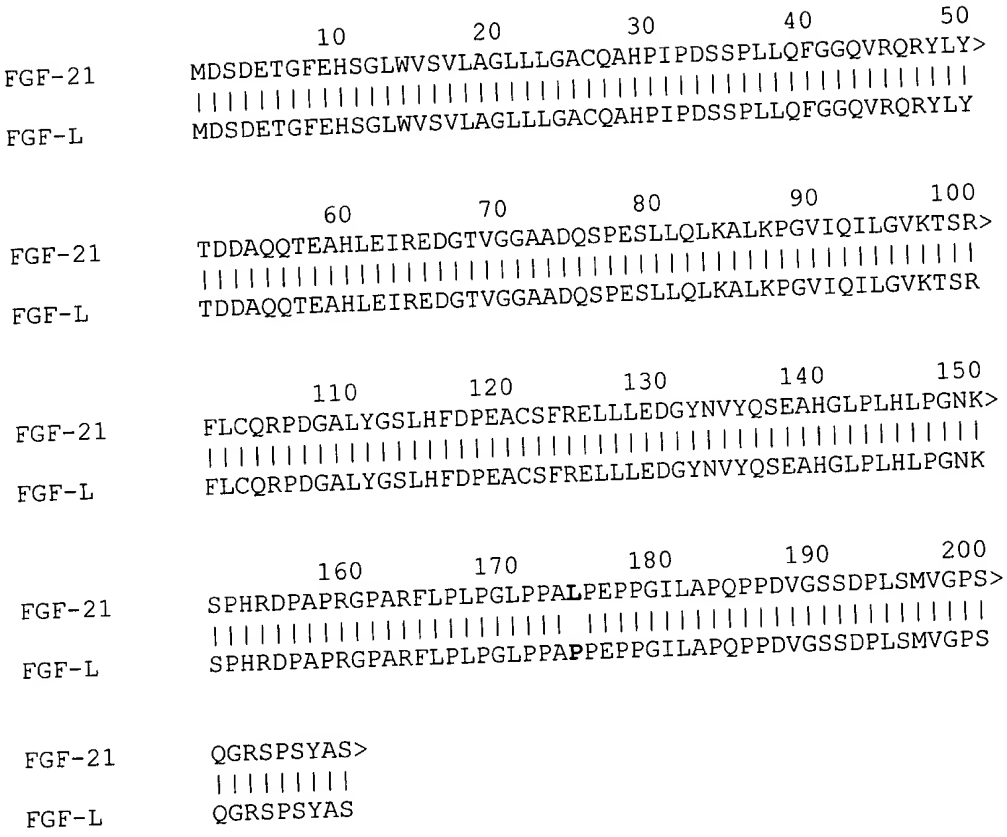
Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.
RID: 991513812-4429-4865
Query=
(209 letters)

Database: nr
693,878 sequences; 218,617,282 total letters

Sequences producing significant alignments:	(bits)	Value
gi|9506597|ref|NP_061986.1| fibroblast growth factor 21 [Homo sa...	329	2e-89
gi|9910218|ref|NP_064397.1| fibroblast growth factor 21 [Mus mus...	257	1e-67
gi|4826726|ref|NP_005108.1| fibroblast growth factor 19 [Homo sa...	85	6e-16
gi|12083589|ref|NP_073148.1| fibroblast growth factor 23 [Mus mu...	85	8e-16
gi|11643230|gb|AAG39478.1|AF315355_1 (AF315355) fibroblast growt...	76	5e-13
gi|6679777|ref|NP_032029.1| fibroblast growth factor 15 [Mus mus...	75	6e-13
gi|10190674|ref|NP_065689.1| fibroblast growth factor 23 [Homo s...	74	1e-12
gi|1345996|sp|P48803|FGF4_BOVIN FIBROBLAST GROWTH FACTOR-4 PRECU...	64	2e-09
gi|817955|emb|CAA35925.1| (X51552) fibroblast growth receptor [M...	64	2e-09
gi|1169677|sp|P21658|FGF6_MOUSE FIBROBLAST GROWTH FACTOR-6 PRECU...	64	2e-09
gi|666915|gb|AAA62261.1| (M92416) fibroblast growth factor [Mus ...	64	2e-09
gi|10337587|ref|NP_066276.1| fibroblast growth factor 6 [Homo sa...	64	2e-09
gi|11436742|ref|XP_007055.1| fibroblast growth factor 6 [Homo sa...	64	2e-09
gi|2118526|pir||JC4268 fibroblast growth factor 4 - bovine	63	5e-09
gi|1346001|sp|P48805|FGFA_XENLA FIBROBLAST GROWTH FACTOR-4-I PRE...	62	5e-09
gi|4503701|ref|NP_001998.1| fibroblast growth factor 4 (heparin ...	62	8e-09
gi|4467836|emb|CAB37648.2| (X14071) FGF.6 protein [Homo sapiens]	61	1e-08
gi|4885233|ref|NP_005238.1| fibroblast growth factor 3 (murine m...	61	1e-08
gi|6679783|ref|NP_032033.1| fibroblast growth factor 3 [Mus musc...	61	2e-08
gi|1346002|sp|P48806|FGFB_XENLA FIBROBLAST GROWTH FACTOR-4-II PR...	60	2e-08
gi|1345997|sp|P48804|FGF4_CHICK FIBROBLAST GROWTH FACTOR-4 PRECU...	60	3e-08
gi|10179934|gb|AAG13950.1| (AF283555) fibroblast growth factor 4...	59	4e-08
gi|309237|gb|AAA37619.1| (M30642) K-fibroblast growth factor [Mu...	59	6e-08
gi|4090567|gb|AAC98812.1| (U76998) putative fibroblast growth fa...	58	8e-08
gi|1345994|sp|P48802|FGF3_BRARE FIBROBLAST GROWTH FACTOR-3 PRECU...	58	1e-07
gi|6753852|ref|NP_034332.1| fibroblast growth factor 4 [Mus musc...	58	1e-07
gi|1091234|prf||2020426A fibroblast growth factor 4 [Gallus gallus]	57	2e-07
gi|3980190|emb|CAA76422.1| (Y16850) fibroblast growth factor 6-r...	56	5e-07
gi|11559990|ref|NP_071547.1| fibroblast growth factor FGF-5 [Rat...	55	6e-07
gi|6753854|ref|NP_034333.1| fibroblast growth factor 5 [Mus musc...	55	6e-07
gi|544288|sp|P36386|FGF3_XENLA FIBROBLAST GROWTH FACTOR-3 PRECUR...	54	2e-06
gi|69035|pir||TVHUF5 fibroblast growth factor 5 - human >gi|1825...	54	2e-06
gi|13637763|sp|P12034|FGF5_HUMAN FIBROBLAST GROWTH FACTOR-5 PREC...	54	2e-06
gi|13630661|ref|XP_003444.2| fibroblast growth factor 5 [Homo sa...	54	2e-06
gi|4758370|ref|NP_004455.1| fibroblast growth factor 5; fibrobla...	54	2e-06
gi|13447396|ref|NP_085113.1| fibroblast growth factor 20 [Mus mu...	53	3e-06
gi|2911146|dbj|BAA24945.1| (D86333) fibroblast growth factor 10 ...	52	4e-06
gi|462249|sp|P34004|FGF1_MESAU HEPARIN-BINDING GROWTH FACTOR 1 P...	52	6e-06
gi|442658|pdb|1BAR|B Chain B, Acidic Fibroblast Growth Factor (A...	52	6e-06
gi|6753846|ref|NP_034330.1| fibroblast growth factor 13 [Mus mus...	52	7e-06
gi|2444477|gb|AAB71606.1| (AF020737) fibroblast growth factor-re...	52	7e-06
gi|10281219|gb|AAG15492.1|AF271786_1 (AF271786) fibroblast growt...	52	7e-06
gi|9789947|ref|NP_062825.1| fibroblast growth factor 20 [Homo sa...	52	7e-06
gi|13027408|ref|NP_076451.1| fibroblast growth factor 20 [Rattus...	52	8e-06

gi|1345995|sp|P48801|FGF3_CHICK FIBROBLAST GROWTH FACTOR-3 PRECU...	52	8e-06
gi|13399478|pdb|1G82|A Chain A, Structure Of Fibroblast Growth F...	51	2e-05
gi|4503707|ref|NP_002001.1| fibroblast growth factor 9 (glia-act...	51	2e-05
gi|6753850|ref|NP_034327.1| fibroblast growth factor 1 [Mus musc...	51	2e-05
gi|544291|sp|P36364|FGF9_RAT GLIA-ACTIVATING FACTOR PRECURSOR (G...	51	2e-05
gi|7305057|ref|NP_038546.1| fibroblast growth factor 9; glia act...	51	2e-05
gi|6911131|gb|AAF31397.1|AF199610_1 (AF199610) fibroblast growth...	51	2e-05
gi|4758360|ref|NP_004456.1| fibroblast growth factor 10 [Homo sa...	51	2e-05
gi|6978837|ref|NP_037083.1| fibroblast growth factor 10 [Rattus ...	51	2e-05
gi|11093923|gb|AAG29501.1| (AF292104) FGF-16 protein [Mus musculus]	50	2e-05
gi|13449275|ref|NP_085117.1| fibroblast growth factor 16 [Mus mu...	50	2e-05
gi|4503691|ref|NP_003859.1| fibroblast growth factor 16 [Homo sa...	50	2e-05
gi|7106313|ref|NP_032028.1| fibroblast growth factor 10 [Mus mus...	50	2e-05
gi|13626683|sp|P79150|FGF7_CANFA KERATINOCYTE GROWTH FACTOR PREC...	50	3e-05
gi|6679785|ref|NP_032034.1| fibroblast growth factor 7 [Mus musc...	50	3e-05
gi|6980585|pdb|1QQK|A Chain A, The Crystal Structure Of Fibrobla...	50	3e-05
gi|11139056|gb|AAG31597.1| (AF295300) FGF7/KGF [Rattus norvegicus]	50	3e-05
gi|7438522|pir||S26049 fibroblast growth factor 7 precursor - ra...	50	3e-05
gi|1346000|sp|P48808|FGF7_SHEEP KERATINOCYTE GROWTH FACTOR PRECU...	49	4e-05
gi|7512161|pir||JC7082 fibroblast somatotropin-20 - African claw...	49	5e-05
gi|442620|pdb|1AFC|A Chain A, Acidic Fibroblast Growth Factor (A...	49	5e-05
gi|4503705|ref|NP_002000.1| fibroblast growth factor 7 (keratino...	49	5e-05
gi|430968|gb|AAA67335.1| (U01670) human keratinocyte growth fact...	49	5e-05
gi|11177916|ref|NP_068639.1| Fibroblast growth factor 16 [Rattus...	49	6e-05
gi|1345990|sp|P48800|FGF2_CHICK HEPARIN-BINDING GROWTH FACTOR 2 ...	49	7e-05
gi|477336|pir||A48834 basic fibroblast growth factor - chicken	49	7e-05
gi|7546630|pdb|1DZC|A Chain A, High Resolution Structure Of Acid...	49	7e-05
gi|8569355|pdb|1EVT|A Chain A, Crystal Structure Of Fgf1 In Comp...	49	7e-05
gi|4503697|ref|NP_000791.1| fibroblast growth factor 1 (acidic) ...	49	7e-05
gi|11513769|pdb|1E0O|A Chain A, Crystal Structure Of A Ternary F...	49	7e-05
gi|226785|prf||1605206A acidic fibroblast growth factor [Homo sa...	49	7e-05
gi|3114266|pdb|2AXM|A Chain A, Heparin-Linked Biologically-Activ...	49	7e-05
gi|7546475|pdb|1DZD|A Chain A, High Resolution Structure Of Acid...	49	7e-05
gi|13236891|gb|AAB29057.2| (S67291) acidic fibroblast growth fac...	49	7e-05
gi|6980644|pdb|1DJS|B Chain B, Ligand-Binding Portion Of Fibrobl...	48	8e-05
gi|122747|sp|P12226|FGF2_XENLA HEPARIN-BINDING GROWTH FACTOR 2 P...	48	9e-05
gi|122734|sp|P03968|FGF1_BOVIN HEPARIN-BINDING GROWTH FACTOR 1 P...	48	9e-05
gi|224785|prf||1201195A fibroblast growth factor,acidic [Bos tau...	48	9e-05
gi|2494456|sp|Q91875|FGF9_XENLA GLIA-ACTIVATING FACTOR PRECURSOR...	48	9e-05
gi|6708457|gb|AAF25944.1|AF213396_1 (AF213396) fibroblast growth...	48	1e-04
gi|4758366|ref|NP_004105.1| fibroblast growth factor 13 [Homo sa...	48	1e-04
gi|4323515|gb|AAD16401.1| (AF100144) fibroblast growth factor 13...	48	1e-04
gi|8132119|gb|AAF73225.1|AF152586_1 (AF152586) acidic fibroblast...	47	1e-04
gi|1345989|sp|P20002|FGF1_PIG HEPARIN-BINDING GROWTH FACTOR 1 PR...	47	1e-04
gi|13626616|sp|Q9N198|FGF7_PIG KERATINOCYTE GROWTH FACTOR PRECUR...	47	2e-04
gi|1909|emb|CAA78854.1| (Z15154) basic fibroblast growth factor ...	47	2e-04
gi|1345991|sp|P48798|FGF2_MONDO HEPARIN-BINDING GROWTH FACTOR 2 ...	47	2e-04
gi|422712|pir||S31622 basic fibroblast growth factor - short-tai...	47	2e-04
gi|1421146|pdb|1BFC| Basic Fibroblast Growth Factor Complexed...	47	2e-04
gi|493887|pdb|1BFG| Basic Fibroblast Growth Factor Mutant Wit...	47	2e-04
gi|1943571|pdb|1BLA| Basic Fibroblast Growth Factor (Fgf-2) M...	47	2e-04
gi|442659|pdb|1BAS| Basic Fibroblast Growth Factor (Bfgf) Mut...	47	2e-04
gi|8569347|pdb|1EV2|A Chain A, Crystal Structure Of Fgf2 In Comp...	47	2e-04
gi|4512022|gb|AAD21576.1| (AF108755) fibroblast growth factor 13...	47	2e-04
gi|6911116|gb|AAF31390.1|AF199602_1 (AF199602) fibroblast growth...	46	3e-04
gi|4512020|gb|AAD21575.1| (AF108754) fibroblast growth factor 12...	46	3e-04

EXHIBIT B


10 20 30 40 50
FGF-21 MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLY>
FGF-L MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLY
60 70 80 90 100
FGF-21 TDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSR>
FGF-L TDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSR
110 120 130 140 150
FGF-21 FLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNK>
FGF-L FLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNK
160 170 180 190 200
FGF-21 SPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPS>
FGF-L SPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQPPDVGSSDPLSMVGPS
FGF-21 QGRSPSYAS>
FGF-L QGRSPSYAS

ELSEVIER

Exhibit C

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Biochimica et Biophysica Acta 1492 (2000) 203–206

www.elsevier.com/locate/bba

Short sequence-paper

Identification of a novel FGF, FGF-21, preferentially expressed in the liver[1]

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Received 11 January 2000; accepted 2 March 2000

Abstract

We isolated cDNA encoding a novel FGF (210 amino acids) from mouse embryos. As this is the 21st documented FGF, we tentatively term it FGF-21. FGF-21 has a hydrophobic amino terminus (~30 amino acids), which is a typical signal sequence, and appears to be a secreted protein. The expression of FGF-21 mRNA in mouse adult tissues was examined by Northern blotting analysis. FGF-21 mRNA was most abundantly expressed in the liver, and also expressed in the thymus at lower levels. We also isolated human cDNA encoding FGF-21 (209 amino acids). Human FGF-21 is highly identical (~75% amino acid identity) to mouse FGF-21. Among human FGF family members, FGF-21 is most similar (~35% amino acid identity) to FGF-19.

Keywords: FGF-21; Fibroblast growth factor; Liver; Gene family; cDNA

The prototypic fibroblast growth factors (FGFs), FGF-1 (aFGF) and FGF-2 (bFGF), originally isolated as mitogens for fibroblasts from the brain and pituitary, are widely expressed in developing and adult tissues [1,2]. The FGF family now consists of 19 published members [1–16]. FGF-3 (int-2), FGF-4 (hst/kFGF), FGF-5 and FGF-6 were identified as oncogene products [5–8]. FGF-7 (KGF) was isolated as a mitogen for cultured keratinocytes [9]. FGF-8 and FGF-9 were isolated as an androgen-induced growth factor and a glia-activating factor from mouse mammary carcinoma cells and human glioma cells, respectively [3,4]. FGF-10 was identified from rat lung by homology-based polymerase chain reaction (PCR) [10]. FGF homologous factors (FHFs), FHF-1 (FGF-12), FHF-2 (FGF-13), FHF-3 (FGF-11) and FHF-4 (FGF-14), were identified from human retina by a combination of random cDNA sequencing, DNA data base searches and homology-based PCR [11]. FGF-15 was identified as a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1 [12]. FGF-16, FGF-17 and FGF-18 were also identified from rat heart and embryos by homology-based PCR [13–15]. FGF-19 was identified by searching DNA data bases [16]. FGF-20 was also identified from rat and human brain by homology-based PCR (The nucleotide sequence data of rat and human FGF-20 cDNA will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with accession numbers AB020021, AB030648; S. Ohmachi et al., submitted for publication). These FGFs, which are expressed during embryonic development and in restricted adult tissues, also play crucial roles in multiple physiological functions including angiogenesis, mitogenesis, pattern formation, cellular differentiation, metabolic regulation and repair of tissue injury [17,18]. Recently, we have identified a novel member, the 21st documented, of the FGF family from mouse embryos by homology-based PCR. Here, we report the structure and expression of FGF-21.

DNA was amplified from mouse embryo cDNA by polymerase chain reaction (PCR) for 30 cycles in 25 μl of a reaction mixture containing each of the sense and antisense degenerate primers representing all possible codons corresponding to the amino acid sequences of human FGF-19, RPDGYN and LPMLPM, respectively [16]. The amplified product was further amplified by PCR with each of the sense and antisense degenerate primers representing all possible codons corresponding to the amino acid sequences of human FGF-19, RPDGYN and HFLPML, respectively [16]. The amplified DNAs of the expected size (approximately 120 bp) were cloned. By determination of the nucleotide sequences of the cloned DNAs, we iden-

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E-mail: itohnobu@pharm.kyoto-u.ac.jp

[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with accession numbers AB021975 and AB025718.

0167-4781/00/$ – see front matter
PII S0167-4781(00)00067-1

```
mouse FGF-21  MEWMRSRVGTLGLWVRLLLAVFLLGVYQAYPIPDSSPLLQFGGQVRQRYLYTDDDQDTEA  60
              *          ****   **  ***  ** ************************* * ***
human FGF-21  MDSDETGFEHSGLWVS-VLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEA  59

              HLEIREDGTVVGAAHRSPESLLELKALKPGVIQILGVKASRFLCQQPDGALYGSPHFDPE  120
              ********** ***  ****** *************** ****** ******** *****
              HLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPE  119

              ACSFRELLLEDGYNVYQSEAHGLPLRLPQKDSPNQDATSWGPVRFLPMPGLLHEPQDQAG  180
              ************************** **   **  *    ** **** ***       *
              ACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPG  179

              FLPPEPPDVGSSDPLSMVEPLQGRSPSYAS   210
               * * ************* * **********
              ILAPQPPDVGSSDPLSMVGPSQGRSPSYAS   209
```

Fig. 1. Amino acid sequence comparison of mouse and human FGF-21. Numbers refer to the amino acid positions of mouse and human FGF-21. Asterisks indicate identical amino acid residues.

tified a novel mouse FGF cDNA. To determine the entire coding region of the novel FGF cDNA, the 5′- and 3′-coding regions were amplified from mouse embryo cDNA by adapter-ligation mediated PCR [19] using a Marathon cDNA amplification kit (Clontech, Palo Alto, CA) and primers specific for the FGF. The nucleotide sequence revealed a complete amino acid sequence of the protein (210 amino acids) (Fig. 1). As this protein is the 21st documented FGF, we tentatively termed it FGF-21. FGF-21 has a hydrophobic amino terminus (~30 amino acids), which is a typical signal sequence, and appears to be a secreted protein. The signal sequence cleavage site of mouse FGF-21 was predicted to lie between amino acid residues 29 (alanine) and 30 (tyrosine) by the method described by Nielsen et al. [20]. Two cysteine residues are well conserved in the FGF family, and these amino acids correspond to residues 60 and 122 in mouse FGF-21. Although a cysteine residue was found at position 122, an alanine instead of a cysteine residue was found at position 60 (Fig. 1).

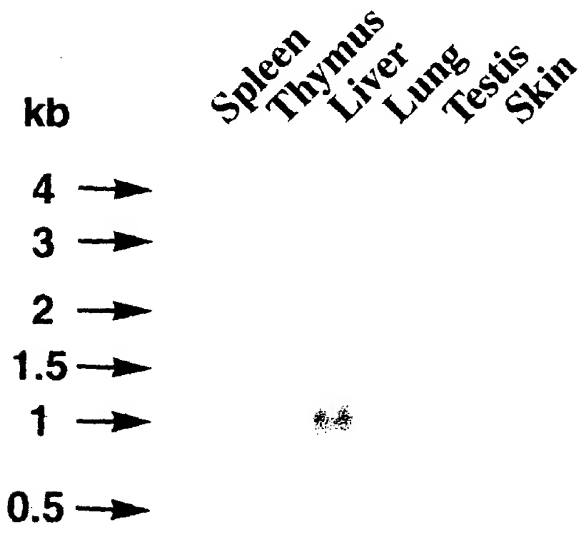

Fig. 2. Expression of FGF-21 mRNA in mouse adult tissues. A nylon filter blotted with mouse tissue poly(A)$^+$ RNAs was hybridized with a ^{32}P-labeled mouse FGF-21 cDNA.

The expression of FGF-21 mRNA in mouse adult tissues was examined by PCR using mouse FGF-21-specific primers and mouse adult tissue cDNAs (brain, heart, liver, kidney, spleen, lung, stomach, small intestine and thymus) (OriGene, Maryland) as templates. FGF-21 mRNA was most abundantly expressed in the liver, and was also expressed in the thymus at lower levels (data not shown). In other tissues, the expression of FGF-21 mRNA was not detected (data not shown). We also examined the expression of FGF-21 mRNA in mouse tissues by Northern blotting analysis. A nylon filter blotted with mouse tissue (spleen, thymus, liver, testis, skin) poly(A)$^+$ RNAs (a Multiple Choice Northern Blot, OriGene) was hybridized with a ^{32}P-labeled mouse FGF-21 cDNA. A strong signal of FGF-21 mRNA (~1000 nucleotides) was detected in the liver (Fig. 2). A weaker signal was also detected in the thymus. In other tissues, there was essentially no signal detected. These results are essentially consistent with PCR findings.

By homology searching in the GenBank with the amino acid sequence of mouse FGF-21, we found that the human FGF-21 gene was located in the 5′-flanking region of human α1,2-fucosyltransferase gene (accession number AB006136) [21]. The cDNA encoding the entire coding region of human FGF-21 was amplified from fetal brain cDNA by PCR using FGF-specific primers including the 5′- and 3′-noncoding sequences, and cloned into the pGEM-T DNA vector. The nucleotide sequence revealed a complete amino acid sequence of human FGF-21 (209 amino acids) (Fig. 1). The amino acid sequence of human FGF-21 is highly identical (~75% amino acid identity) to that of mouse FGF-21. Among FGF family members, human FGF-21 is most similar (~35% amino acid identity) to human FGF-19 [16] (Fig. 3). The apparent evolutionary relationships of 21 members of the FGF family were examined using a sequence analysis software, Genetyx (Software Development, Tokyo, Japan). FGF-21 was found to be closest to FGF-19 (Fig. 4).

```
Human FGF-21    MDSDETGFEHSGLWVSVLAGLLLG-ACQAHPIPDSSPLLQF--GGQVRQRYLYTDDAQQ-  56
                         *  **** * *       * *     *  * * ***
Human FGF-19    MRSGCVVVHVW--ILAGLWLAVAGRPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGL   55

                TEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHF 116
                   * ** ** *  *  **  ***  **       * **  * **  **  * *
                SSCFLRIRADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQY 115

                DPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPH--RDPAPRGPARFLPLPGLPPAL 174
                  * * * *    ****** ** * **  *   *                ***    *
                SEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEE 175

                PEP-PGILAPQ----PPDVGSSDPLSMV-GPSQGRSPSYAS  209
                **   * *       *   * **  * *   ****
                PEDLRGHLESDMFSSPLETDSMDPFGLVTGLEAVRSPSFEK  216
```

Fig. 3. Amino acid sequence comparison of human FGF-21 with human FGF-19. Numbers refer to the amino acid positions of human FGF-21 and human FGF-19. Asterisks indicate identical amino acid residues.

In conclusion, we identified a novel FGF, FGF-21, which is most similar (~35% amino acid identity) to FGF-19 among members of the FGF family. FGF-21 mRNA was preferentially expressed in the liver, although no FGF other than FGF-21 has been reported to be preferentially expressed in the liver. FGFs are local signal molecules that act on proximal cells [2]. Therefore, FGF-21 is expected to be a unique FGF that plays important roles in the liver.

This work was supported in part by the Yamada Science Foundation, the Yamanouchi Foundation on Metabolic Disorders, a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, and the Human Frontier Science Program, France.

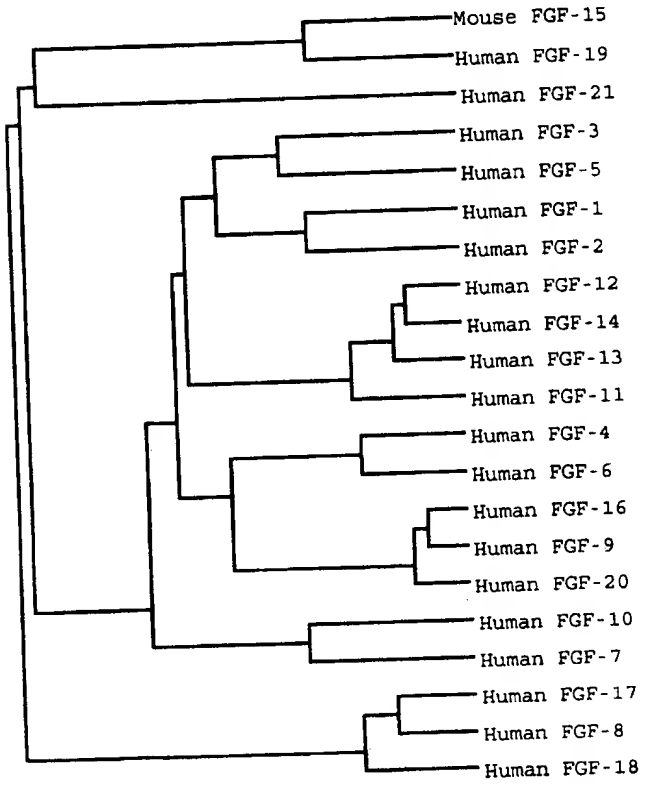

Fig. 4. Apparent evolutionary relationships of 21 members of the FGF family. The length of each horizontal line is proportional to the degree of amino acid sequence divergence

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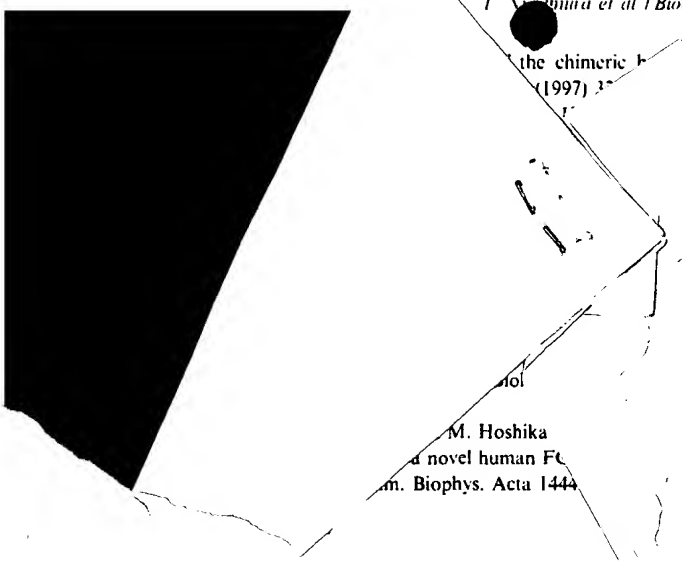

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ATCC

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Amgen Inc.
Attn: Benxian Liu
One Amgen Center Drive
Thousand Oaks, CA 91320

Deposited on Behalf of: Amgen Inc.

Identification Reference by Depositor:	**Patent Deposit Designation**
cDNA in *Escherichia coli* DH10B, pSPORT/FGF-like	PTA-626

The deposit was accompanied by: ___ a scientific description _X_ a proposed taxonomic description indicated above.

The deposit was received September 3, 1999 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested September 16, 1999. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Deposit ry

Date: September 17, 1999

cc: Robert B. Winter

BP4/9

AMENDMENTS TO THE SPECIFICATION

Marked Up Versions of Amended Paragraphs of the Specification under 37 C.F.R. 1.121(b)(1)(iii)

Please amend the paragraph at page 2, line 11 to page 3, line 14 as follows:

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c) a nucleotide sequence encoding a polypeptide that is at least about 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4 wherein the polypeptide activates one or more FGF receptors, regulates the growth and differentiation of cells within the liver, regulates other cell types following secretion from the liver, plays a role in liver chemotaxis, has an oncogenic activity, or serves as an antigen for generating antibodies;

(d) an allelic variant or splice variant of any of (a), (b) or (c);

(e) the nucleotide sequence of the DNA insert in ATCC Deposit No. [_____]PTA-626;

(f) a nucleotide sequence of (b), (c), or (d) encoding a polypeptide fragment of at least about 25 amino acid residues wherein the polypeptide fragment activates one or more FGF receptors, regulates the growth and differentiation of cells within the liver, regulates other cell types following secretion from the liver, plays a role in liver chemotaxis, has an oncogenic activity, or serves as an antigen for generating antibodies;

(g) a nucleotide sequence of (a), (b), or (c) comprising a fragment of at least about 16 to 18 nucleotides;

(h) a nucleotide sequence encoding a polypeptide that has a substitution and/or deletion of 1 to 100 amino acid residues in the amino acid sequence as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 wherein the polypeptide activates one or more FGF receptors, regulates the

growth and differentiation of cells within the liver, regulates other cell types following secretion from the liver, plays a role in liver chemotaxis, has an oncogenic activity, or serves as an antigen for generating antibodies; [and]

(i) a nucleotide sequence which hybridizes under stringent conditions to the complement of any of (a) - (h); and

(j) a nucleotide sequence complementary to any of (a), (b), (c), or (i).

Please amend the paragraph at page 3, line 16 to page 4, line 4 as follows:

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(b) the mature amino acid sequence as set forth in SEQ ID NO: 5 or SEQ ID NO: 6 comprising a mature amino terminus at residue 29 in the mature human amino acid sequence and at residue 30 in the mature mouse amino acid sequence, optionally further comprising an amino-terminal methionine;

(c) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 comprising at least about 25 amino acid residues wherein the fragment activates one or more FGF receptors, regulates the growth and differentiation of cells within the liver, regulates other cell types following secretion from the liver, plays a role in liver chemotaxis, has an oncogenic activity, or serves as an antigen for generating antibodies;

(d) the amino acid sequence encoded by the DNA insert of ATCC Deposit No. [_____]PTA-626;

(e) an ortholog of SEQ ID NO: 2 or SEQ ID NO: 4; and

(f) an allelic variant or splice variant of (a), (b), (d), or (e).

Please amend the paragraph at page 8, lines 7-13 as follows:

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The term "FGF-like nucleic acid molecule" refers to a nucleic acid molecule comprising or consisting essentially of a nucleotide sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3, comprising or consisting essentially of a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, comprising or consisting essentially of a nucleotide sequence of the DNA insert in ATCC Deposit No. [_____]PTA-626, or nucleic acid molecules related thereto.

Please amend the paragraph at page 71, lines 23-26 as follows:

A deposit of cDNA encoding FGF-like polypeptide in *E. coli* strain [_____] DH10B has been made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on [_____]September 3, 1999 and having accession No. [_____]PTA-626.

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AMENDMENTS TO THE CLAIMS

Marked Up Versions of Amended Claims under 37 C.F.R. 1.121(c)(1)(ii)

1. (Twice Amended) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c) the nucleotide sequence of the DNA insert in ATCC Deposit No. [_____]PTA-626;

(d) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (c); and

(e) a nucleotide sequence complementary to any of (a) - (c).

2. (Amended) A recombinant host cell comprising a nucleic acid molecule comprising the nucleotide sequence of Claims 1, 39, or 40.

12. (Amended) A process for determining whether a compound inhibits FGF-like polypeptide activity or FGF-like polypeptide production comprising exposing a cell according to Claim[s] 2[, 3, 4, 8, 9, or 10] to the compound, and measuring FGF-like polypeptide activity or FGF-like polypeptide production in said cell.

13. (Amended) A process for producing a protein comprising growing a culture of the host cell of Claim[s] 9[, 10, or 11] in suitable culture medium and isolating the protein from the culture.

36. (Twice Amended) A method of modulating levels of an FGF-like polypeptide in an animal comprising administering to the animal the nucleic acid molecule of Claims 1, 39, or 40[, or 8].

39. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide that is at least about 80 percent identical to the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4 wherein the polypeptide has an activity of the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4, or serves as an antigen for generating antibodies;

(b) a nucleotide sequence encoding an allelic variant or splice variant of either the nucleotide sequence as set forth in either SEQ ID NO: 1 or SEQ ID NO: 3; the nucleotide sequence of the DNA insert in ATCC Deposit No. [_____]PTA-626; or (a) wherein the polypeptide has an activity of the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4;

(c) a nucleotide sequence of either SEQ ID NO: 1 or SEQ ID NO: 3; the DNA insert in ATCC Deposit No. [_____]PTA-626; (a); or (b); encoding a polypeptide fragment of at least about 25 amino acid residues wherein the polypeptide fragment has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or serves as an antigen for generating antibodies;

(d) a nucleotide sequence of either SEQ ID NO: 1 or SEQ ID NO: 3; the DNA insert in ATCC Deposit No. [_____]PTA-626; or (a) - (c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (d) and wherein the polypeptide has an activity of the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4; and

(f) a nucleotide sequence complementary to any of (a) - (c).

40. (Amended) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

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(b) a nucleotide sequence encoding a polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(c) a nucleotide sequence encoding a polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(d) a nucleotide sequence encoding a polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(e) a nucleotide sequence encoding a polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 4;

(f) a nucleotide sequence of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (f); and

(h) a nucleotide sequence complementary to any of (a) - (e).

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